ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
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in its capacity as elected Office

Date of mailing (day/month/year) 30 August 2000 (30.08.00)	
International application No. PCT/US99/26724	**Applicant's or agent's file reference** 32014-PCT
International filing date (day/month/year) 10 November 1999 (10.11.99)	**Priority date** (day/month/year) 12 November 1998 (12.11.98)
Applicant HAYES, Mark, A. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 May 2000 (17.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
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Form PCT/IB/331 (July 1992)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

4

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
32014-PCT		
International application No. PCT/US99/26724	International filing date *(day/month/year)* 10 NOVEMBER 1999	Priority date *(day/month/year)* 12 NOVEMBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): G01N 27/477 and US Cl.: 204/454,601		
Applicant ARIZONA BOARD OF REGENTS		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17 MAY 2000	22 FEBRUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN S. STARSIAK JR. DEBORAH THOMAS PARALEGAL SPECIALIST Telephone No. (703) 308-0661

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/26724

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-38, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the claims:
pages 39-42, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the drawings:
pages 1-4, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/26724

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-16	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	6 and 12	YES
	Claims	1-5,7-11,13-16	NO
Industrial Applicability (IA)	Claims	1-16	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-4,8-10,13-16 lack an inventive step under PCT Article 33(3) as being obvious over Ghowsi in view of Ewing et al. Ghowsi discloses all the particular recited in the above claims except that Ghowsi is silent concerning the cross-section of the flow channel. Ghoswi teaches [col.5, lines 57-66]: "The thickness of the insulator is determined by the range of voltages which may be applied. Uniformity of thickness is desirable. There are competing considerations in determining the optimal thickness of the insulator layer. On the other hand, *the thinner the layer, the smaller the voltage needed*. On the other hand, it is difficult to fabricate very thin capillaries with uniform thickness, and such thin capillaries tend to be fragile. A compromise between these competing consideration must be reached. A preferred range for the insulator thickness in the case of silica will be 1-100 micron. The range 40-100 micron is practical to fabricate by traditional means; below that range may be achieved by silicon micromachining techniques". Ghowsi teaches [col. 8, lines 8-11]: "One possibility is that of performing multiple capillary electrophoresis on a single chip, having several capillaries "tuned" to different species, and acting simultaneously in parallel.". Ghowsi teaches [col. 6, line 14]: "V_G ranges from -300 to +300 volts...". Ewing et al discloses a device similar to Ghowsi with a flow channel diameter of 20 microns. It would have been obvious to one of ordinary skill in the art at the time of the invention to made the flow channel of Ghowsi 20 microns in diameter because this value is typical for the art.

Claims 5 and 11 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Young et al.

Young et al discloses a device similar to Ghowsi and Ewing in which the conductive coating was applied to the fused silica capillary without removing the polyimide coating (i.e. commercially available fused silica capillaries are normally (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
coated with polyimide). Hence, it would have been obvious to one of ordinary skill in the art at the time of the invention that the device of Ghowsi could be fabricated from a fused silica capillary as supplied by the manufacturer, i.e. not necessary to remove polyimide coating).

Claim 7 lacks an inventive step under PCT Article 33(3) as being obvious over Ghowsi in view of Ewing et al and Blanchard et al.

For the modification of Ghowsi in view of Ewing et al see first rejection. Ghowsi is silent concerning coating the interior of the flow channel. Blanchard et al teaches {col. 4, lines 4-8]; "Because the process employed using the apparatus reduces or substantially eliminates interior wall adsorption of molecules, no interior coating need be used, but of course, if the specific application contemplated requires such a coating, it can be applied.". It would have been obvious to one of ordinary skill in the art at the time of the invention to coat the interior of the flow channel if the electrophoretic separation process requires it as taught by Blanchard et al.

Claims 6 and 12 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest a device for performing fluid flow comprising: a substrate defining a capillary channel, wherein the capillary channel comprises an inner wall surface, two ends, and a cross section of less than about 200 times 10^{-9} square meters; at least one integrated external electrode spaced apart from the inner wall surface of the capillary channel by a distance d of less than about 160 times 10^{-6} meters, wherein the integrated external electrode is positioned to provide a perpendicular voltage field to the capillary channel; two longitudinal electrodes, one of said longitudinal electrodes being positioned at one end of the capillary and the other of said longitudinal electrodes being positioned at the other end of the capillary channel, wherein the longitudinal electrodes are positioned at the intermediate ends of the capillary channel and are positioned to provide a longitudinal voltage field selectively through the capillary channel; and one of the following particulars: 1) further comprising a means for real-time flow measurement with feedback for monitoring and controlling the flow of fluids in the capillary channel, 2) further comprising titanium dioxide positioned between at least one of the integrated external electrodes and the capillary channel. The devices of claims 6 and 12 would be useful for performing capillary electrophoresis.

---------- NEW CITATIONS ----------

US 5,151,164 A (BLANCHARD et al) 29 September 1992, col.6, lines 5-9.

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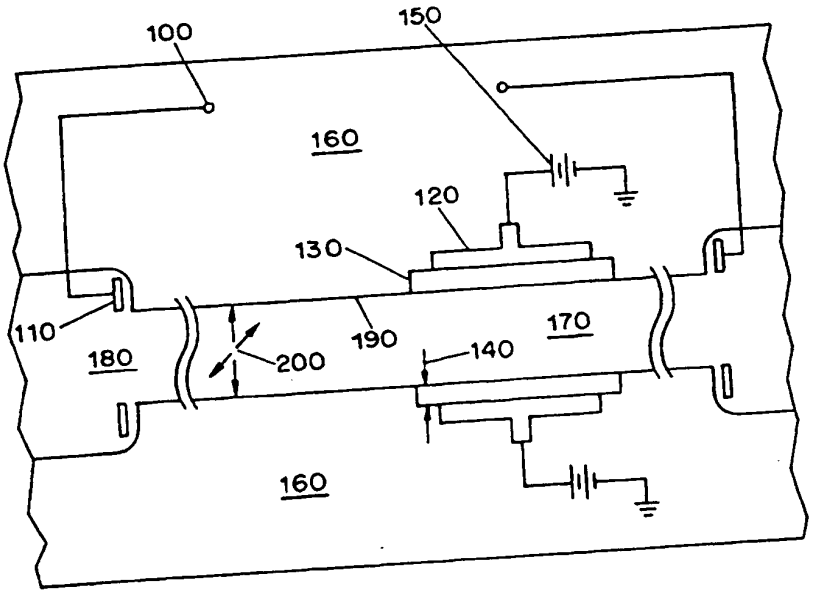

(57) Abstract

A device for control of ultrasmall volume fluid flow used in the fields of electrophoretic separation, chemical analysis, and microchemical reactions has a substrate defining a capillary channel and integrated external electrodes to control electroosmotic flow. The channel geometry and integrated external electrode proximity reduce the voltage required for control of flow. Longitudinal electrodes provide electrophoretic separation of components. High dielectric material between the integrated external electrode and capillary reduces the voltage required for the control of flow. Real-time flow monitoring and capillary channel surface coating enhance the control of flow.

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PRACTICAL DEVICE FOR CONTROLLING ULTRASMALL VOLUME FLOW

SPECIFICATION

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority of United States Provisional Application No. 60/108,086, filed November 12, 1998, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD OF THE INVENTION

This invention relates to the fields of electroosmosis and electrophoresis, and in particular to a device for controlling the movement of fluids in a capillary channel used in chemical systems for separations, reactions, or analysis.

BACKGROUND ART

Microdevices for fluids. Movement of fluids on microchips has been accomplished by a number of methods. Most notably by pneumatic pressure and electroosmosis, as described in Seiler, et al., Analytical Chemistry 1994, 66, 3485-3491, which is hereby incorporated by reference in its entirety. Pressure-induced flow generally requires physical valves to be fabricated and placed in the flow stream. This must be done to control the variety of fluid movements needed on complex

microdevices. However, these valves are difficult to design and fabricate, and exhibit poor back-pressure and leakage performance, as described in Manz, et al., Sensors and Actuators 1990, B1, 244-248, which is hereby incorporated by reference in its entirety. Also, the valves have not been fabricated on the micron to sub-micron scale that are required for future generations of microdevices. Even if these physical structures could be fabricated reliably with good performance characteristics, pressure-induced flow does not scale down very well to narrow passages and ultrasmall volumes. The back pressure generated by these minute passages is immense and the size of the valve structures lead to dead volume and time delays in flow between volume elements.

Electroosmosis is the most important flow-generating mechanism, which originates at the solution/wall interfacial region. Immediately adjacent to the solid-solution interface, the so-called double layer is formed, as described in Davies, et al., Interfacial Phenomena, 2nd ed., Academic Press, New York, 1963, which is hereby incorporated by reference in its entirety. Under most normal aqueous buffer conditions, a silica wall surface has an excess negative charge. This results from chemical ionization of surface functional groups. This negatively charged surface attracts buffer counter ions which collect near the surface in a complex layered system. This action creates a potential across these layers, where the potential dropped across the diffuse layer is termed the ζ(zeta)-potential. The ζ-potential is dependent upon the viscosity of the fluid, the dielectric constant of the solution and the charge on the inner surface of the wall of the capillary. The cationic counter ions

(H_3O^+, Na^+ typically) entrained in the diffuse layer are free to migrate towards the anode, and because these ions are solvated, they drag solvent with them. The ζ-potential and the longitudinal electric field strength governs the rate of flow, as described in Rice, et al., Phys. Chem. 1965, 69, 4017, which is hereby incorporated by reference in its entirety.

In the field of microfabricated devices, remarkable progress has been made in miniaturization of separation-based systems. In 1992, Manz, et al. and Harrison, et al., first established the use of a separation system on a microfabricated device, as described in Manz, et al., Journal of Chromatography 1992, 593, 253-258, and Harrison, et al., Analytical Chemistry 1992, 64, 1926-1932, which are hereby incorporated by reference in their entirety. Efforts have continued to optimize and miniaturize a wide variety of analytical based separation systems, as described in Effenhauser et al., "Integrated chip-based capillary electrophoresis," Electrophoresis 1997, 18, 2203-2213, which is hereby incorporated by reference in its entirety. The development of such devices has drawn emphasis to the field of small volume fluid manipulation. Electroosmosis (also termed electroosmotic flow) provides an efficient means of fluid flow control in a network of interconnecting channels. This flow generates a flat-flow profile regardless of shape and dimension of the channel, thus minimizing dispersion within the system. Since electroosmosis is directly proportional to the applied longitudinal voltage field, the control of flow in each channel is effected by varying its potential gradient. Flow in interconnecting channels can be controlled by applying voltages in accordance to a model based on Kirchoff's

law where the various channels are treated as homogeneous resistors in an electrical network, as described in Seiler, et al., Analytical Chemistry 1994, 66, 3485-3491.

While electroosmosis provides a near-ideal flow propulsion mechanism on microdevices, in practice it has proven difficult to apply reliably and, as practiced in present systems, has some inherent limitations, as described in Effenhauser, et al., Electrophoresis 1997, 18, 2203-2213. The mechanisms which generate electroosmosis are complex, involving an interplay between surface composition and buffer characteristics. Since it is an interfacial phenomenon, minute amounts of materials depositing (or leaving) the surface can create dramatic changes in this flow. This has resulted in poor reproducibility in standard separation techniques and made microfluidic flow control problematic. For instance, to apply the flow control model according to Kirchoff's law, the ζ-potential, ionic strength and the buffer pH in all channels must be kept constant. Another limitation of using electroosmosis as a propulsion mechanism is that both the flow rate and the electrophoretic migration rates of charged species are directly coupled to the voltage field strength. In standard systems the flow rate cannot be independently varied without unduly influencing the movement of charged species.

Electroosmosis is also an important component of capillary zone electrophoresis, which is a powerful separation technique characterized by high-efficiency, low volume separations, as described in Beale, Analytical Chemistry 1998, 70, 279R-300R and P. Camilleri, Capillary Electrophoresis Theory and Practice, 2nd ed.; CRC Press: New York, 1998, which are hereby incorporated by

reference in their entirety. This technique can be used to separate both charged and neutral analytes in a wide variety of applications, including amino acids, proteins, and nucleic acids. The flow generated is usually large enough to force all species present (cations, anions, and neutrals) to migrate in one direction allowing the analysis of all species at a single detector. Electroosmosis directly influences the efficiency, resolution and reproducibility of electrokinetic separation techniques. Capillary electrophoresis and its ancillary techniques have also been demonstrated for a number of different applications on microdevice formats, as described in Effenhauser et al., Electrophoresis 1997, 18, 2203-2213.

Electroosmosis can be altered in a variety of ways. Examples of purposefully altering electroosmotic flow (EOF) include buffer additives, as described in Jorgenson, et al., Science 1983, 222, 266-272; Hjerten, Chromatogr. 1985, 347, 191-198 and Bruin, et al., Chromatogr. 1989, 471, 429-436 altering buffer pH, as described in Lukacs, et al., J. High Res. Chrom. & Chrom. Comm. 1985, 8, 407-411; Lambert, et al., Analytical Chemistry 1990, 62, 1585-1587 and McCormick, Analytical Chemistry 1988, 50, 2322-2328 altering buffer concentration, as described in Lukacs, et al., J. High Res. Chrom. & Chrom. Comm. 1985, 8, 407-411; Issaq, et al., Chromatographia 1991, 32, 155-161; Atamna, et al., J. Liq. Chromatogr. 1990, 13(16); 3201-3210 and Atamna, et al., J. Liq. Chromatogr. 1990, 13, 2517-2527 coating the inner wall of the capillary, as described in Jorgenson, et al., Science 1983, 222, 266-272; Hjerten, Chromatogr. 1985, 347, 191-198 and Moseley, et al., Analytical Chemistry 1991, 63, 109-114 and organic modifiers, as described in

VanOrman, et al., J. Microcol. Sep. 1990, 2, 176-180 and Schwer, et al., Analytical Chemistry 1991, 63, 1801-1807, which are hereby incorporated by reference in their entirety. These techniques either (1) permanently alter the surface structure, or (2) alter the buffer composition. They result in a static, new rate of electroosmotic flow (EOF) which cannot actively be altered in response to changing conditions with the channel or tube. However, dynamic control of electroosmosis has been predicted and demonstrated by applying an additional radial voltage field across the wall of the capillary (for fused silica capillaries used in conventional capillary electrophoresis), as described in Lee, et al., Analytical Chemistry 1990, 62, 1550-1552; Lee, et al., Analytical Chemistry 1991, 63, 1519-1523; Huang, et al., "Mechanistic Studies of Electroosmotic Control at the Capillary-Solution Interface," Analytical Chemistry 1993, 65, 2887-2893; Hayes, et al., "Electroosmotic Flow Control and Monitoring with an Applied Radial Voltage for Capillary Zone Electrophoresis," Analytical Chemistry 1992, 64, 512-516, and Hayes, et al., "Effects of Buffer pH on Electroosmotic Flow Control by an Applied Radial Voltage for Capillary Zone Electrophoresis," Analytical Chemistry 1993, 65, 27-31, which are hereby incorporated by reference in their entirety. The radial voltage flow control technique does not require permanent changes in surface structure, or altered buffers. This control effectively decouples the electrophoretic migration of charged species and the bulk flow rate.

Thus, it would be beneficial to provide an apparatus for controlling the flow of ultrasmall fluid volumes of the kind used in microchips and microdevices for the chemical, biochemical, and analytical sciences.

Radial voltage flow control. Radial voltage flow control, a method to control electroosmotic flow, was first demonstrated using resistive solutions or materials covering the majority of the outer surface of the capillary, as described in Lee, et al., Analytical Chemistry 1990, 62, 1550-1552. This design required resistive materials so that radial potential matched the potential gradient of the buffer on the interior of the capillary (offset by the radial voltage experimental value). Later work demonstrated that the effect could be generated by conductive materials or ionized gas and that the matching of the interior potential gradient was unimportant in obtaining the effect, as described in Hayes, et al., Analytical Chemistry 1993, 65, 27-31 and Wu, et al., "Dispersion Studies of Capillary Electrophoresis with Direct Control of Electroosmosis," Analytical Chemistry 1993, 65, 568-571, which is hereby incorporated by reference in its entirety. In fact, control was demonstrated while covering only very small portions (4%) of the outer surface with a conductor, as described in Hayes, et al., "Electroosmotic Flow Control and Surface Conductance in Capillary Zone Electrophoresis," Analytical Chemistry 1993, 65, 2010-2013, which is hereby incorporated by reference in its entirety. Surface conductance within the electric double layer was attributed for the effective control where the induced charge from the radial voltage spread along the inner surface. This charge affected the ζ-

potential over the entire capillary length effectively inducing the change in electroosmosis.

Investigations of this effect have demonstrated some limitations to this technique. The radial voltage cannot manipulate flow in standard fused silica capillaries with buffer pH above approximately 5, as described in Hayes, et al., Analytical Chemistry 1993, 65, 27-31. High ionic strength buffers have also been predicted to limit its effectiveness. Additional dispersion is predicted from the heterogeneous ζ-potential caused by radial fields in the partially covered capillaries. This has been the subject of several theoretical discussions, but has yet to be experimentally confirmed, as described in Potocek, et al., Journal of Chromatography 1995, 709, 51-62; Keely, et al., J. Chromatogr. A 1993, 652, 283-289; Cortes, et al., J. Microcol. Sep. 1989, 1, 278-288; Keely, et al., Analytical Chemistry 1994, 66, 4236-4242; Kasicka, et al., Journal of Chromatography 1997, 772, 221-230; Anderson, et al., Chem. Engin. Commun. 1985, 38, 93-106 and Chien, et al., Analytical Chemistry 1991, 63, 1354-1361, which are hereby incorporated by reference in their entirety. Radial voltage flow control also requires very large voltages, at least several to many kilovolts, to generate the radial fields in fused silica capillaries. These large electrical potentials have presented severe design limitations, and safety and expense problems for the application of this technique.

For example, Ghowsi disclosed in U.S. Patent No. 5,092,972 that radial voltage flow control could be done at lower voltages in a silica capillary of up to 100 micrometer wall thickness, in which radial voltage differences could be applied

uniformly across the entire length of the capillary. However, no capillary channel cross section was disclosed.

In another example, Blanchard et al. disclosed in U.S. Patent No. 5,151,164 a radial voltage flow control device using a fused silica capillary of 530 micrometer inside diameter having an inner capillary of 75 micrometer outside diameter (channel cross section 216 x 10^{-9} square meters of the annular region between the capillaries) and 630 micrometer outside diameter in which radial voltage differences of 5 to 6 kilovolts were applied across the annular region between the capillaries to halt electroosmotic flow. The distance between the radial voltage electrode and the annular channel inner wall surface was 100 micrometers.

In another example, Young et al. disclosed in U.S. Patent No. 5,180,475 a radial voltage flow control device using a fused silica capillary of 50 micrometer inside diameter (channel cross section 2 x 10^{-9} square meters) and from 140 to 360 micrometer outside diameter, in which radial voltage differences of 5 kilovolts were applied across the capillary to control electroosmotic flow. The distance between the radial voltage electrode and the channel inner wall surface was from 45 to 155 micrometers.

In a further example, Ewing et al. disclosed in U.S. Patent No. 5,320,730 a radial voltage flow control device using a fused silica capillary of either 20 micrometer inside diameter (channel cross section 0.3 x 10^{-9} square meters) and 144 micrometer outside diameter, or 50 micrometer inside diameter (channel cross section 2 x 10^{-9} square meters) and 370 micrometer outside diameter, in which radial

voltages of up to 30 kilovolts were applied across the capillary to control electroosmotic flow. The distances between the radial voltage electrode and the channel inner wall surfaces were 62 and 160 micrometers, respectively.

In a recent example, Ewing et al. disclosed in U.S. Patent No. 5,358,618 a radial voltage flow control device using a fused silica capillary of 20 micrometer inside diameter (channel cross section 0.3×10^{-9} square meters) and 144 micrometer outside diameter, in which radial voltages of up to 30 kilovolts were applied across the capillary to control electroosmotic flow. The distance between the radial voltage electrode and the channel inner wall surface was 62 micrometers.

However, the above-mentioned patents fail to disclose devices or methods that allow efficient control of electroosmotic flow for ultrasmall fluid volumes with reduced perpendicular voltage fields.

SUMMARY OF THE INVENTION

Therefore, it is an object of the present invention to provide a device and method for producing reliable electroosmotic flow of a fluid in a capillary channel with dynamic control using an external voltage field that is applied in an orientation perpendicular to the capillary channel. It is another object of the present invention to provide a device and method for efficient control of electroosmotic flow of a fluid in a capillary channel with a reduced perpendicular voltage applied. A further object of the invention is to provide a device and method for monitoring uniform electroosmotic flow of a fluid.

These objectives have been substantially satisfied and the shortcomings of the prior art have been substantially overcome by the present invention, which in one embodiment is directed to a capillary channel device having an integrated external electrode positioned microscopically close to a capillary channel of ultrasmall cross section, wherein the overall geometry increases the channel inner wall surface charge density produced by a particular strength of the perpendicular voltage field. The microscopic distance between the integrated external electrode, which provides the perpendicular voltage field, combined with the ultrasmall cross section of the capillary channel reduces the voltage required for electroosmotic flow control.

In another embodiment, the present invention is directed to a capillary channel device having an integrated external electrode positioned microscopically close to a capillary channel of ultrasmall cross section, wherein longitudinal electrodes are positioned at the immediate ends of the channel to apply a longitudinal voltage field selectively within the channel to induce electrophoretic migration of substances within the channel. This embodiment permits independent control of the bulk fluid flow and the electrophoretic migration, and permits selective control of the flow in each channel when a plurality of channels are combined in a device.

In another embodiment, the present invention is directed to a capillary channel device having an integrated external electrode positioned microscopically close to a capillary channel of ultrasmall cross section, wherein a material of high dielectric constant is positioned between the integrated external electrode and the

capillary wall to inject charge to the capillary channel inner wall surface when voltage is applied to the integrated external electrode. This embodiment further reduces the voltage required for electroosmotic flow control, and reduces the effect that the perpendicular voltage field applied to one channel has on other nearby channels when a plurality of channels are combined in a device.

In a further embodiments, the present invention is directed to a capillary channel device as described above, further comprising a means to monitor the flow of fluids in the capillary channel, and optionally having an inner channel wall surface coating to enhance control of fluid flow.

BRIEF DESCRIPTION OF THE DRAWINGS

Further objects, features, and advantages of the present invention will be more fully appreciated from a reading of the detailed description when considered in conjunction with the accompanying drawings, wherein:

Fig. 1 illustrates an example of a device for ultrasmall volume flow control according to a preferred embodiment of the present invention;

Fig. 2 illustrates the physical parameters of geometry of a device for ultrasmall volume flow control according to a preferred embodiment of the present invention;

Fig. 3 illustrates a plot of model capillary inner wall surface charge density versus internal and external diameter from an applied external perpendicular voltage;

Fig. 4 illustrates an example of a microchip device for ultrasmall volume fluid flow control according to a preferred embodiment of the present invention;

Fig. 5. illustrates a plot of fluorescent intensity of a dye migrating through a microchip channel versus time at various applied perpendicular voltages;

Fig. 6. illustrates an example of a device for ultrasmall volume flow control according to a preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

By "integrated external electrode" we mean an electrical conductor positioned with respect to the capillary channel so that a potential applied to the integrated external electrode will produce a perpendicular voltage field with respect to the capillary channel.

By "perpendiclar voltage field" we mean the component of the electric field emanating from the integrated external electrode which is in a direction perpendicular to the capillary channel, and the electric charges produced anywhere within the device by the application of an electric potential to the integrated external electrode, whether produced directly by the field or indirectly by conduction or other movement of charge. The perpendiclar voltage field provides control of electroosmotic flow in the capillary channel.

The present invention provides a practical device for controlling ultrasmall volume fluid movements using reduced voltages to control electroosmotic

flow, as shown in Fig. 1. Fluid flow is provided in a capillary channel **170** defined by a substrate **160**, wherein the channel has two ends **180**. The voltage field used to control electroosmotic flow is applied perpendicularly across a capillary channel having an ultrasmall cross section **200**, wherein the distance **140** between the perpendicular field-generating integrated external electrode **120** and the capillary channel inner wall surface **190** is microscopically small. A means **150** for applying a voltage to the integrated external electrode is provided. In one embodiment, a material of high dielectric constant **130** is positioned between the integrated external electrode and the capillary channel, and optionally the material of high dielectric constant may form a portion of the capillary channel inner wall surface. Longitudinal electrodes **110** are provided at the immediate ends **180** of the capillary channel to effect electrophoretic migration and electroosmosis of fluids within the channel. The longitudinal electrodes can be electrically connected to nodes **100** for connecting to a means for applying a voltage difference between the two longitudinal electrodes, or optionally the longitudinal electrodes **110** can be adjacent to, and in electrical contact with an object outside the device that provides a voltage difference between the two longitudinal electrodes.

This device will find application, for example, with capillary zone electrophoresis. Another example is any fluid movement within microinstrumentation driven by electrokinetic effects, including instrumentation and methods for separation science. Both of these applications involve the transport and/or storage of fluids for

chemical reactions or analysis. All of these examples will benefit from the processes described in this disclosure.

The device can be made as a microchip, as shown in Fig. 2. The capillary channel **170** is again defined by the substrate **160**, and can have ultrasmall cross sectional dimensions **200**. Integrated external electrodes **120** can be positioned a microscopically small distance **140** from the capillary channel. The substrate of the device can be a ceramic, silica, fused silica, quartz, a silicate, a titanate, a metal oxide, a nitride, silicon, titanium dioxide, and the like, or a polymer, a plastic, a polydimethylsiloxane, or a polymethylmethacrylate.

Microscopic distances between integrated external electrodes and the channel. Without intending to be bound by any one particular theory, the equation describing the physical and electrical properties of the capillary channel predicts that reduced distances between the integrated external electrodes and the channel wall will enhance the efficiency of electroosmotic flow control, where control is done with the perpendicular voltage effect, by a factor of $1/\ln(r_o/r_i)$, as shown in Fig. 3, and as described in Hayes, et al., Analytical Chemistry 1992, 64, 512-516. The reduced distances will have an additional benefit: the applied voltages may be lower in absolute magnitude, thus reducing technological requirements for insulation and safety. The two limitations for applying this concept are: the structural integrity of the wall and the electrical breakdown of the insulating (or wall) material. Through careful design, these limitations are minimized.

The first issue is structural integrity. The electric double layer surface conductance, mentioned briefly above, aids in the design of this system, as described in Wu, et al., Analytical Chemistry 1993, 65, 568-571, and Hayes, et al., Analytical Chemistry 1993, 65, 2010-2013. Surface conductance provides a mechanism for the charge to spread out along the length of the capillary channel on the inner wall surface. Earlier studies have shown that the charge created by a perpendicular voltage in one limited section of the capillary channel can affect the double layer throughout the entire length of the capillary channel, and is able to effectively control flow. The electrode (or conductor, more accurately) could be placed very near (nanometers to microns) to the capillary channel inner wall surface of this segment.

Electrical breakdown is not an issue. As the initial surface charge density increases, the induced surface charge becomes less and less effective in changing the flow in this system, as described in Huang, et al., Analytical Chemistry 1993, 65, 2887-2893 and Hayes, et al., Analytical Chemistry 1993, 65, 27-31. The maximum effective surface charge on the inner surface will be obtained at voltages well below the electrical breakdown voltage limit. This is especially true for the high dielectric constant materials. The additional induced charge becomes ineffective in changing the flow at high positive or negative values. This limit is obtained at approximately 2.1×10^{13} charges/cm^2. Experimentally, this charge may be induced across a 10 micrometer silicate wall with 350 volts with only 17% of the calculated electrical breakdown voltage for this thickness. It is even more favorable with a

titanium dioxide wall, where only 8.8 V is required, which is only 0.4% of the breakdown voltage.

Channels fabricated with reduced distances between the integrated external electrodes and the channel wall dramatically improve control of electroosmosis. A small portion of the device used to demonstrate this is shown in Fig. 4, wherein a microchip capillary channel device is illustrated. The microchip substrate **160** defines a capillary channel **170**. Two integrated external electrodes **120** are positioned at a reduced distance **140** of 50 micrometers from the capillary channel and its inner wall surface **190**. A material of high dielectric constant **130** can be positioned between the integrated external electrodes and the channel wall. Injection can be done with a standard offset cross-tee design and detection can be done with laser induced fluorescence. With this device the control of electroosmosis is accomplished at perpendicular voltages that are ten to one hundred times less than conventional systems, as shown in Fig. 5, wherein the elution data for the device indicate that using reduced distances between the integrated external electrodes and the channel wall for a capillary channel of ultrasmall cross section results in dramatically improved control of electroosmosis with less demanding power supplies. We have also demonstrated that electrical breakdown is not a problem with this design. To construct the device as a microchip or microdevice, other fabrication techniques are used, including chemical vapor deposition and alternative materials.

Ultrasmall capillary channel cross section. A fused silica capillary tube may be modeled as a cylindrical capacitor, as described in Keely, et al., J.

Chromatogr. A 1993, 652, 283-289. Without intending to be bound by any one particular theory, in this model the surface-charge density (q) created on the inner surface by an applied radial voltage has a relationship with the physical properties as follows:

$$q = \epsilon_q V_r(1/r_i)(1/\ln(r_o/r_i)) \quad (2)$$

where ϵ_q is the permittivity of the fused silica, V_r is the applied radial voltage, r_i is the inner radius, and r_o is the outer radius. This equation indicates that at very small inner diameters the efficiency of the applied radial voltage is maximized.

The quantitative improvements predicted are illustrated by graphing inner diameter versus radial voltage-induced inner surface charge density, as shown by the x-axis versus y-axis, respectively, in Fig. 3. Compared to previously used experimental radii of about 10 to 75 micrometers inside diameter (i.d.) and 150 to 360 micrometers outside diameter (o.d.), the charge density, as shown in Fig. 3, increases by several orders of magnitude, as described in Hayes, et al., Analytical Chemistry 1993, 65, 27-31 and Wu, et al., Analytical Chemistry 1993, 65, 568-571. This change of the geometry of the capillary channel provides for improved control of electroosmosis.

Materials with high dielectric constant. Materials which make up the capillary channel wall can also improve the control of flow. They can increase the effectiveness of flow control by inducing a greater amount of charge on the inner surface of the channel for a given applied radial voltage. This higher induced charge, in turn, induces greater affect on the ζ-potential, thereby improving the control of

flow. A material with high dielectric constant (the ϵ_q term in eqn. 2), such as titanium dioxide, can be positioned between the integrated external electrode and the channel to serve this purpose. The typical substrate material, quartz (or fused silica) has a dielectric constant of 3.8, whereas that of titanium dioxide has been reported to be as much as 170. The amount of charge transferred to the channel inner wall surface is linear with the permittivity (as indicated by the dielectric constant). Thus, by using a material of high dielectric constant, up to 40 times more electric charge will be injected to the channel inner wall surface for a given applied perpendicular voltage field, all other factors being equal. Other materials useful for the high dielectric material are ceramics, a silicate, a titanate, a metal oxide, a nitride, titanium dioxide, and the like, or a high dielectric polymer or plastic.

The direction in which the electric charge is transferred can be controlled by using high dielectric constant materials because charge is more effectively transferred across high dielectric constant materials, than the substrate. The position of the integrated external electrode with respect to the high dielectric material can be used to inject charge in a particular direction in the device. By placing a high dielectric material between an integrated external electrode and the channel, the charge will be preferentially injected towards the channel. In one embodiment, wherein the device is a combination of capillary channels each with perpendicular voltage flow control, as shown in Fig. 6, controlling the direction of electric charge from the integrated external electrodes is particularly important. As shown in Fig. 6, it prevents the perpendicular voltage field from one integrated

external electrode **120** from influencing the flow in nearby channels **170**. As devices become smaller and more complex, both the channels and the flow control mechanisms will necessarily be in closer proximity. Thus, it will become increasingly important to reduce the influence of one integrated external electrode on the flow in adjacent channels. By inducing charge preferentially in one direction, the effects on nearby channels are minimized. The amount of charge injected to the desired channel versus that injected to a nearby channel is the result of two properties: (1) the ratio of the distance between neighboring channels and the distance between the integrated external electrode and the channel wall, and (2) the dielectric constant of the material positioned between the integrated external electrode and its channel wall (for example, TiO_2), and the dielectric constant of the substrate material between the integrated external electrode and the nearby channel (for example, SiO_2). The ratio of the distances can be 100 or more, and the ratio of the dielectric constants can be as high as 40. Thus, the discrimination between changes in flow produced in the desired channel by the integrated external electrode and changes of flow unintentionally produced in nearby channels can be as high as 4000, even for complex microchip devices.

Channel inner wall surface coating. An optimal inner-surface coating for the perpendicular voltage flow control of electroosmosis requires three properties. First, the surface must retain low surface charge density in the presence of the aqueous buffers typically used in capillary electrophoresis, as described in Poppe, et al., Analytical Chemistry 1996, 68, 888-893, and Hayes, "Extension of External

Voltage Control of Electroosmosis to High-pH Buffers," Analytical Chemistry 1999, 71, 3793-3798. Second, the surface charge density should be insensitive to pH changes of the buffer, thus remaining consistent over a large range of normally encountered pH (for example, pH from 1 to 11) and buffer types, as described in Hayes, et al., Analytical Chemistry 1993, 65, 27-31. Finally, the surface must not increase the solution viscosity near the surface, as described in Huang, et al., J. Microcol. Sep. 1992, 4, 135-143, and Huang, et al., J. Chromatogr. A 1994, 685, 313-320. The viscosity within the electric double layer defines the frictional forces retarding the entrained ions movement in the longitudinal voltage gradient and has a direct effect on electroosmotic mobility. High-viscosity surface layers produce low electroosmosis altogether, as described in Manz, et al., Sensors and Actuators 1990, B1, 244-248, and Jorgenson, et al., Science 1983, 222, 266-272. Avoiding the use of polymers or polymer-forming reactants, or the use of monolayer surface coverage minimizes increases in local viscosity.

A variety of coatings fulfill these criteria. Notably, silicate surfaces treated with hindered organosilanes and ceramic oxide surfaces (TiO_2, for example) with organosilane treatments. The silicate surface is labile to acid and base degradation reactions, but with the hindered organosilane treatment this surface remains stable up to eight weeks, as described in Hayes, "Extension of External Voltage Control of Electroosmosis to High-pH Buffers," Analytical Chemistry 1999, 71, 3793-3798. Coating silicate with titanium dioxide and then reacting that surface with organosilanes forms an uncharged, stable surface, as described in Pesek, et al.,

Chromatographia 1997, 44, 538-544, which is hereby incorporated by reference in its entirety. The organosilane coating on the titanium dioxide does not require hindered reagents since the underlying material is not liable to the acid and base degradation reactions. Any additional coatings which meet the criteria listed above will function to aid the flow-control system.

The surface charge generated by the chemical equilibrium of buffer/wall interface must be minimized to extend radial voltage flow control to higher buffer pH, as described in Hayes, et al., Analytical Chemistry 1993, 65, 27-31, and Poppe, et al., "Theoretical Description of the Influence of External Radial Fields on the Electroosmotic Flow in Capillary Electrophoresis," Analytical Chemistry 1996, 68, 888-893, which is hereby incorporated by reference in its entirety. This has generally been accomplished with surface coatings, which are described here.

Coatings constructed with polymers eliminate the chemical equilibrium-based surface charge and increase local viscosity, as described in Srinivasan, et al., Analytical Chemistry 1997, 69, 2798-2805; Huang, et al., J. Microcol. Sep. 1992, 4, 135-143; and Huang, et al., J. Chromatogr. A 1994, 685, 313-320, which are hereby incorporated by reference in their entirety. They are designed to minimize protein adsorption and eliminate or permanently change electroosmosis. Polymers have been covalently bound and physically adsorbed to the inner wall surface of the capillary channel or used as dynamic coatings (where buffer additives with surface-active properties adhere to the wall in a adsorbed/free-solution equilibrium), as described in Srinivasan et al., Analytical Chemistry 1997, 69, 2798-

280, and Iki et al., J. Chromatogr. A 1996, 731, 273-282, which is hereby incorporated by reference in its entirety. These polymers suppress electroosmosis by reduced surface charge density and increased viscosity within the electric double layer. This local viscosity is unaffected by the perpendicular voltage potential gradients which alter electroosmosis, and therefore polymer coatings are unacceptable for dynamic flow control by an applied perpendicular field, as described in Huang et al., Analytical Chemistry 1993, 65, 2887-2893.

Fused-silica capillaries coated with organosilane treatments have been reported, most notably for application to capillary gas chromatography. However, due to the labile silicon/oxygen/carbon bond system, previous organosilane treatments were not stable at buffer pH extremes, either high or low, as described in Srinivasan, et al., Analytical Chemistry 1997, 69, 2798-2805; Hjerten, et al., Electrophoresis 1993, 14, 390-395; and Kirkland, et al., Analytical Chemistry 1989, 61, 2-11, which are hereby incorporated by reference in their entirety.

Organosilane treatments have also been explored for perpendicular voltage flow control for capillary electrophoresis. One example was the use of commercially 'deactivated' tubing (the surface treatment was proprietary, but was known to be organosilane based), where the authors merely mention that it "... yields effective EOF [electroosmotic flow] control by applied radial voltage," without further explanation, as described in Hayes, et al., Analytical Chemistry 1992, 64, 512-516. A butylsilane surface was also used to improve the effectiveness of flow control, but the surface was unstable above pH 5, as described in Huang, et al., Analytical

Chemistry 1993, 65, 2887-2893, and Towns, et al., J. Chromatogr. 1990, 516, 69-78, which is hereby incorporated by reference in its entirety. Sterically hindered triorganosilane treatments have demonstrated stability to acidic and basic buffers and provided for perpendicular voltage flow control from pH 2 to pH 10, as described in Hayes, "Extension of External Voltage Control of Electroosmosis to High-pH Buffers," Analytical Chemistry 1999, 71, 3793-3798, which is hereby incorporated by reference in its entirety.

Flow monitoring. The flow rate and direction of flow for each channel can be monitored. This information is used as a feedback mechanism to confirm or to appropriately adjust the flow control mechanisms. The rate of flow will be adjusted according to the information provided by the monitor. One requirement of this monitoring device is that the materials and fluid within the channel must remain unchanged by the monitoring system. The monitoring system must be non-invasive because any disturbance of the condition or make-up of fluid contained within the channel may preclude its use in subsequent operations. Any flow monitoring system which can detect flow rates non-invasively in microns-wide channels will function for the technology described here.

A summary of methods for real-time monitoring of electroosmosis prior to 1989 is given in Goor, et al., J. Chromatogr. 1989, 470, 95-104, which is hereby incorporated by reference in its entirety. The first and most commonly applied of these methods is the use of a neutral marker, as described in Lukacs, et al., J. High Res. Chrom. & Chrom. Comm. 1985, 8, 407-411; Lauer, et al., Analytical Chemistry

1986, 58, 166-170; and Stevens, Analytical Chemistry 1983, 55, 1365-1370, which are hereby incorporated by reference in their entirety. In capillary electrophoresis, neutral species are swept along at the electroosmotic flow rate (in the absence of surface interactions). Therefore, if the length from the injector to the detector is known, the flow may be calculated from the elution time. This technique is limited to monitoring only the average flow during the analysis.

Streaming potential has been used to determine the ζ-potential where the flow is calculated from this value, as described in Rutgers, et al., in Physical Chemistry: enriching topics from colloid and surface science, edited by Olphen and Mysels; Theorex, La Jolla, California, 1975; Hunter, Zeta Potential in Colloid Science, Principles and Applications, Academic Press, London, 1981; Wegenen, et al., J. Colloid Interface Sci. 1980, 76, 305; Wegenen, et al., J. Electrochem. Soc. 1976, 123, 1438; and Reijenga, et al., J. Chromatogr. 1983, 260, 241, which are hereby incorporated by reference in their entirety. This system requires pressure driven buffer reservoirs and highly sensitive voltage sensing devices. This also requires off-line analysis, from which the flow is back calculated.

One method to directly measure EOF is to weigh the mass transferred from the injection or the mass delivered to the detection reservoir, as described in Goor, et al., J. Chromatogr. 1989, 470, 95-104; Altria, et al., Anal. Proc. 1986, 23, 453-454; and Altria, et al., Chromatographia 1987, 24, 527-532, which are hereby incorporated by reference in their entirety. This of course requires calibration for each

buffer system and a high accuracy mass balance system. In addition, to calculate the linear velocity, the capillary internal diameter must be accurately known.

Monitoring the current flow in a capillary has been used to examine the rate of electroosmosis when a buffer of differing concentration is introduced into the injection end of the capillary, as described in Lee, et al., Analytical Chemistry 1990, 62, 1550-1552; and Huang, et al., Analytical Chemistry 1988, 60, 1837-1838, which is hereby incorporated by reference in its entirety. Under these conditions the total conductivity across the capillary is proportional to a weighted average of the conductivity of each buffer solution. Therefore, the rate of change in the current is a function of the flow rate. Buffers must be changed for each analysis and flow will slightly vary as the capillary fills with a different buffer.

An example of a flow monitoring system that can be used in a preferred embodiment in the present invention is described in Ewing et al., U.S. Patent No. 5,624,539, which is hereby incorporated by reference in its entirety.

Longitudinal electrode positioning. In existing designs, the electrodes which generate the electrokinetic effects are typically placed in buffer or sample reservoirs. In the present invention, electrodes placed at the immediate ends of all channels, or selected channels, allow introduction of an electric field selectively within the channel. Because of their positioning, these longitudinal electrodes provide the option of limiting the electrokinetic effects to the materials and fluids contained only within the channel. Thus, either the electrophoretic migration or the bulk flow may be independently adjusted. Bulk flow can be directly changed by the applied

longitudinal voltage field, or by changes in the ζ-potential caused by perpendicular voltage fields. Electrophoretic migration may be changed by varying the longitudinal voltage field. The manipulations provided for with this device and procedures will allow for precise liquid injection and handling within a microdevice.

The following examples are given to illustrate important features of the present invention and are not intended to limit the invention in any way. It should be understood that the present invention is not limited to the above-mentioned embodiments. Numerous modifications can be made by one skilled in the art having the benefits of the teachings of the present invention. Such modifications should be taken as being encompassed within the scope of the present invention as set forth in the appended claims.

EXAMPLE 1

Reagents. Sodium dihydrogen phosphate (NaH_2PO_4) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin) and was used as received. N-(2-aminoethyl)-4-amino-3,6-disulfo-1,8-naphthalimide, dipotassium salt (lucifer yellow) was obtained from Molecular Probes (Eugene, Oregon) and was used as received. All NaH_2PO_4 buffers were prepared to a 20 mM (millimolar) concentration and adjusted to pH 3.0 using phosphoric acid (EM Science, Gibbstown, New Jersey). Lucifer yellow was prepared (1 mg/mL) using NaH_2PO_4 buffer. All buffers and samples were degassed under vacuum for 5 minutes and were filtered with a Millex-LCR Filter Unit, 0.5 micrometer pore size (Bedford, Massachusetts).

Lucifer yellow solutions were filtered with a Nalgene filter (0.2 micrometer pore size, Fisher Scientific, Pittsburgh, Pennsylvania). All buffers and samples were prepared with 18 megohm purified water drawn from a NANOpure UV ultrapure water filtration system (Barnstead, Dubuque, Iowa).

Planar Microdevice. A capillary channel microdevice was designed in-house and manufactured by the Alberta Microelectronic Centre (Edmonton, Alberta). This device consisted of a long capillary channel, used for electrophoretic separation, intersected by two off-set side channels. The substrate was Corning 0211 glass (Precision Glass and Optics, Santa Ana, California). The overall device measured 2.54 cm x 7.62 cm. The channel dimensions were 30 micrometers wide and 10 micrometers deep. The side channels were off-set by 500 micrometers. The separation channel (injection zone to the buffer waste reservoir) was 5.0 cm long. Integrated external electrodes were positioned parallel to the main channel, separated by 50 micrometers of glass substrate, as shown in Fig. 4. The integrated external electrodes extended 6 mm total, centered at 9 mm from the end of the separation channel. The effective perpendicular voltage field strength was determined by first calculating the potential of the buffer (assuming a linear potential gradient) immediately adjacent to the center of the integrated external electrode, derived from the longitudinal voltage gradient. The effective perpendicular voltage field was the difference between the calculated buffer potential at that point and the potential applied to the integrated external electrode by the power supply means.

Apparatus. Two Series 225 high voltage power supplies were used to apply potential to the longitudinal and integrated external electrodes (Bertran, Hickesville, New York). An Olympus Vanox microscope (Tokyo, Japan) and an Olympus IX70 Inverted Research microscope (Tokyo, Japan) were used for imaging. An Omnichrome Model 100 HeCd laser was used as the fluorescence excitation source (442 nm). Image acquisition was performed with an RS170 CCD camera (CSI Electronics, East Hartford, Connecticut) integrated with National Instruments Lab VIEW IMAQ image acquisition software and hardware (National Instruments, Austin, Texas) where imaging programs were developed in-house. Data analysis was performed using MathCAD 7.0 (MathSoft, Inc., Cambridge, Massachusetts) and Excel (Microsoft Corporation, Seattle, Washington) programs that also were developed in-house.

Results. Dramatically improved efficiency was demonstrated for control of electroosmosis with small applied potentials to the integrated external electrodes of less than about 120 V. Two separate quantitative data sets (normalized and simulated capillary zone electrophoresis) indicated that the system was stable and consistent while providing efficient control. The device was approximately 40 times more efficient than conventional fused silica capillary systems described in the literature to control fluid flow.

Representative digital images were acquired of the flow of a fluorescent sample bolus moving through the capillary channel five seconds after the injection of the sample. Different velocities of the injected bolus were observed under

various effective voltage fields of the integrated external electrodes, as shown in Table I. The change observed in the electroosmotic mobility of the sample for the experiments in which the value of the effective voltage field of the integrated external electrodes was changed from +4 V to +124 V was 8.0×10^{-5} cm^2/Vs. In theory, the maximum change in mobility that could be achieved for this device under these conditions was about 8×10^{-4} cm^2/Vs. Thus, by changing the effective voltage field of the integrated external electrodes by only 120 V, about 10% of the maximum allowable change in sample mobility was obtained. Furthermore, the results in Table I exhibited a linear correlation between the normalized change in observed mobility and the effective voltage field of the integrated external electrodes according to the equation $y = \{-2.13 \times 10^{-3}\}x + 1.07$ (where y is the normalized change in observed mobility, x is the effective voltage, and $R^2 = 0.84$ for the correlation).

Further studies were performed by simulating a capillary zone electrophoresis experiment. The fluorescence intensity was monitored at a pseudo-detection window located approximately 5 mm away from the injector with the CCD camera and software manipulation. Changes in electroosmosis caused by the effective voltage field of the integrated external electrodes were observed in a more conventional manner with this method. A higher voltage gradient between the longitudinal electrodes was used for the electrophoretic separation (-123.9 V/cm), and the injection rate was increased for these experiments to generate shorter run times. The elution time for the fluorescent sample dye varied dramatically with the change in the effective voltage field of the integrated external electrodes. Peak elution times

varied by as much as 16 ± 3 seconds over a 5 mm separation distance, as shown in Table II. In Table II, the observed mobility changed by 7.9×10^{-5} cm^2/Vs for a change in the effective voltage field of the integrated external electrodes of 120 V (from 52 V to 172 V). Thus, the results of the experiments shown in Tables I and II were in agreement, even when the centers of the ranges of effective voltages of the integrated external electrodes that were used were somewhat different (+4 V to +124 V versus +52 V to +172 V). A linear correlation also existed between the observed mobility and the effective voltage fields of the integrated external electrodes for the results in Table II, according to the equation $y = \{6.6 \times 10^{-7}\}x - \{2.1 \times 10^{-4}\}$ (where y is the observed mobility, x is the effective voltage, and $R^2 = 0.94$ for the correlation). The values for observed mobility in Table II were not normalized to initial mobility, as were the values in Table I, thus the slope of the correlation and the magnitude of the values were different in Tables I and II.

The effectiveness of flow control for the microdevice tested here versus standard capillary electrophoresis systems which use fused silica substrates was calculated by comparing the experimental results obtained here to the published literature. This analysis was limited to studies using buffers consistent with those used in this study (pH 3, 1 to 50 mM phosphate). To quantitate the effectiveness of a voltage field of the integrated external electrodes with respect to flow velocity, the following analysis was undertaken, as shown in Table III. First, the total positive range of the applied voltage used to control electroosmosis in the literature reference was listed in Table III. Since the absolute value of the inner and outer radii of the

capillary tubes used in these literature references influenced the effectiveness of the applied radial voltage (see equation 2, above), the applied radial voltage was multiplied by a cylindrical capacitor factor of $1/(r_i \ln(r_o/r_i))$ to obtain the values of effective radial voltage of 109 to 450 V/micrometer in Table III. The corresponding change in the electroosmotic mobility ($\Delta\mu_{eof}$) from the literature references ranged from 8.0×10^{-5} cm^2/Vs to 3.2×10^{-4} cm^2/Vs in Table III. An efficiency factor, Γ, was calculated, where $\Delta\mu_{eof}$ was divided by the applied capacitor field strength, as given by the equation, $\Gamma = \Delta\mu_{eof}/[V_r/(r_i \ln(r_o/r_i))]$. The efficiency factor in Table III varied from 3.7×10^{-7} $(cm^2/Vs)/(V/micrometer)$ to 1.5×10^{-6} $(cm^2/Vs)/(V/micrometer)$.

For the experiments shown in both Tables I and II, the range of applied potential of the integrated external electrodes was 120 V. Since the voltage was applied across a distance of 50 micrometers between the integrated external electrodes and the capillary channel wall, the range of the field gradient that was applied was 2.4 V/micrometer. The corresponding change in the observed electroosmotic mobility was 8×10^{-5} cm^2/Vs. Thus, for the experiments of Tables I and II, the efficiency factor was $\Gamma = 3.3 \times 10^{-5}$, which was 22 times greater than the next highest literature reference value shown in Table III, 43 times greater than the average value of the literature references, and 90 times greater than the lowest value of the literature references. Thus, the efficiency of the microdevice shown here in controlling electroosmotic flow was far greater than for standard capillary electrophoresis systems.

TABLE I

EXTERNAL VOLTAGE EFFECTS ON NORMALIZED OBSERVED MOBILITY*

Effective Voltage of Integrated External Electrodes (V)	Normalized Change in Observed Mobility (exp. value μ_{obs}/initial μ_{obs})	Number of Trials
-26	1.25±0.12	10
4	1.17±0.30	13
14	1.19±0.14	9
34	1.26±0.46	21
44	1.11±0.10	5
64	1.00±0.54	41
84	0.99±0.05	4
104	0.98±0.03	4
124	0.94±0.03	4
144	0.93±0.07	4

* Linear correlation: $y = \{-2.13 \times 10^{-3}\}x + 1.07$ ($R^2 = 0.84$). Note: 0 V applied voltage to the integrated external electrodes results in a 64 V effective field (data were normalized to this value).

TABLE II

CHANGES IN OBSERVED MOBILITY USING INTEGRATED EXTERNAL ELECTRODES**

Effective Voltage of Integrated External Electrodes (V)	Elution Time (s)‡	Observed Mobility (μ_{obs}) (x 10^4 cm^2/Vs)	Number of Trial
52	21.7±0.3	-1.86±0.03	5
72	24.1±3.7	-1.68±0.25	6
112	32.2±2.1	-1.25±0.08	10
152	35.4±2.6	-1.14±0.08	5
172	37.8±4.1	-1.07±0.11	5

** Linear correlation $y = \{6.6 \times 10^{-7}\}x - \{2.1 \times 10^{-4}\}$ ($R^2 = 0.94$).

‡ Data taken 5 mm away from injection zone.

TABLE III

COMPARISON OF EFFICIENCY OF INTEGRATED EXTERNAL ELECTRODES IN MICRODEVICE TO LITERATURE REFERENCES

Reference†	Ionic Strength (mM)‡	Capillary i.d. / o.d. (micrometer)	Range of Radial Voltage (V)	Effective Radial Voltage (V/micrometer)•	Change in Electroosmotic Mobility ($\Delta\mu_{eof}$) (x 10^4 cm^2/Vs)	Efficiency Factor (Γ) [x 10^6(cm^2/Vs)/ (V/micrometer)]
[1](Wu)	10	50/150	3000	109	1.4	1.3
[2](Wu)	10/20	50/150	6000	218	0.80	0.37
[3](Huang)	10	50/150	8000	291	1.5	0.51
[3](Huang)	1	50/150	8000	291	1.4	0.48
[3](Huang)	10	50/150	10000	364	1.9	0.52
[3](Huang)••	10	50/150	5000	182	2.3	1.3
[3](Huang)§	10	50/150	6000	218	0.95	0.43
[4](Hayes)	1	25/144	6000	208	3.2	1.5
[4](Hayes)	1	10/144	6000	450	2.2	0.49

† Data taken directly from reference, or calculated from experimental description.

‡ All buffers were pH 3.

• According to $V_r/(r_i \ln(r_o/r_i))$; see text above and reference for explanation.[5]

•• Capillary coated with an organic phase containing butyl functional groups.

§ Capillary coated with an organic phase containing amino functional groups.

(1) Wu, et al., Analytical Chemistry 1993, 65, 568-571.

(2) Wu, et al., Analytical Chemistry 1992, 64, 2310-2311.

(3) Huang, et al., Analytical Chemistry 1993, 65, 2887-2893.

(4) Hayes, et al., Analytical Chemistry 1993, 65, 27-31.

(5) Hayes, et al., Analytical Chemistry 1992, 64, 512-516.

EXAMPLE 2

Reagents. Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide and anhydrous ethyl alcohol (Aldrich); t-butyldiphenylchlorosilane (United Chemical Technologies, Inc., Bristol, Pennsylvania); and 200 nm carboxylate modified yellow-green fluorescent (505 nm excitation/515 nm emission) latex

microspheres (Molecular Probes, Eugene, Oregon) were used as received. All NaH_2PO_4 buffers were prepared to 100 mM concentration and adjusted with 100 mM sodium hydroxide to pH 5.1. Capillaries were coated by combining 30 microliters of t-butyldiphenylchlorosilane with 1 mL anhydrous ethyl alcohol and pressure rinsing the capillary.

Instrumentation. Capillaries were fused silica (35 and 45 cm in length, 20 micrometers i.d. and 150 micrometers o.d.; Polymicro Technologies, Phoenix, Arizona), where the one tip was sputter-coated with chromium, and then gold, after removing the polyimide coating (Desk II Sputtering Unit, Denton Vacuum Inc., Cherry Hill, New Jersey). Thus, a longitudinal electrode was placed exactly at the end of the capillary channel, and in this example did not occupy any portion of the capillary channel. These tips were physically connected to a platinum electrode which was formed about the circumference of the solution reservoir that was external to the device. The capillary electrophoresis system to which the device was interfaced was built in-house and used a CZE1000R high voltage power supply (Spellman High Voltage Electronics Corporation, Hauppauge, New York); a vacuum pump system (CENCO Hyvac, Fort Wayne, Indiana); a 100 mW He-Cd dual wavelength laser (442 nm/325 nm) (Omnichrome laser, Chino, California); a CC-5E CCD camera (HutchNET, East Hartford, Connecticut); and an Olympus VANOX stereo microscope (Tokyo, Japan). Data collection and analysis programs were developed in-house using LabVIEW software and an IMAQ PCI-1408 image acquisition board

from National Instruments (Austin, Texas). Modeling was accomplished using programs developed in-house using MathCAD 7.0.

The device was interfaced by placing the cathodic buffer reservoir in a sealed plexiglas container where vacuum or pressure could be applied. The anodic buffer reservoir was fashioned from plexiglas material to form a container where the gold-coated capillary tip of the device and the reservoir buffer were maintained at the same potential. This allowed the longitudinal potential field to be initiated at the immediate end of the capillary channel. Data analysis was performed by recording fluorescence intensity near the end of the capillary channel (quantitation was 10 pixels by 500 pixels for 2.5 micrometers by 120 micrometers). The fluorescence was monitored from the carboxylate-modified latex spheres over time as voltage fields were adjusted to balance electrophoretic migration of the microspheres against the bulk inward flow. A cross-sectional 10 pixels were then averaged and analyzed using programs developed in-house using MathCAD 7.0 and Excel (Microsoft) on an Optiplex GXI Pentium 233 (DELL Computer Corporation, Round Rock, Texas).

EXAMPLE 3

A substrate of Corning 0211 glass is fabricated defining a capillary channel 30 micrometers wide by 10 micrometers deep, and 5 cm long, as in Example 1. An integrated external electrode is positioned parallel to the channel separated by 50 micrometers from the channel, and extending longitudinally 1 cm in both directions from the longitudinal center of the channel. A layer of titanium dioxide, a

high dielectric material, is positioned between the integrated external electrode and the channel, extending longitudinally 0.2 cm in both directions from the longitudinal center of the channel. A voltage is applied to the integrated external electrode to directionally inject charge density to the channel wall.

THE INVENTION CLAIMED IS:

1. A device for performing fluid flow comprising:

a substrate defining a capillary channel, wherein the capillary channel comprises an inner wall surface, two ends, and a cross section of less than about 200×10^{-9} square meters; and

at least one integrated external electrode spaced apart from the inner wall surface of the capillary channel by a distance d of less than about 160×10^{-6} meters, wherein the integrated external electrode is positioned to provide a perpendicular voltage field to the capillary channel; and

two longitudinal electrodes, one of said longitudinal electrodes being positioned at one end of the capillary channel and the other of said longitudinal electrodes being positioned at the other end of the capillary channel, wherein the longitudinal electrodes are positioned at the immediate ends of the capillary channel and are positioned to provide a longitudinal voltage field selectively through the capillary channel.

2. The device of claim 1, wherein the distance d is less than about 50×10^{-6} meters.

3. The device of claim 1, wherein the capillary channel cross section is less than about 50×10^{-9} square meters and the distance d is less than about 50×10^{-6} meters.

4. The device of claim 1, wherein the capillary channel cross section is less than about 2×10^{-9} square meters and the distance d is less than about 50×10^{-6} meters.

5. The device of claim 1, further comprising a high dielectric material being positioned between at least one of the integrated external electrodes and the capillary channel.

6. The device of claim 1, further comprising a means for real-time flow measurement with feedback for monitoring and controlling the flow of fluids in the capillary channel.

7. The device of claim 1, further comprising a coating on said inner wall surface.

8. A combination device for performing fluid flow comprising a plurality of devices according to claim 1.

9. A microchip comprising the device according to claim 1.

10. The device of claim 1, wherein said substrate comprises a material selected from the group consisting of ceramics, silica, fused silica, quartz, silicates, titanates, metal oxides, nitrides, silicon, titanium dioxide, polymers, plastics, polydimethylsiloxanes, polymethylmethacrylates, and mixtures thereof.

11. The device of claim 5, wherein said high dielectric material comprises a material selected from the group consisting of ceramics, silicates, titanates, metal oxides, nitrides, polymers, plastics, polydimethylsiloxanes, polymethylmethacrylates, and mixtures thereof.

12. The device of claim 5, wherein the high dielectric material comprises titanium dioxide.

13. An electrophoretic separation process using the device of claim 1, comprising the steps of:

(1) introducing a fluid comprising the species to be separated into the capillary channel;

(2) applying a voltage of less than about 2000 volts to the integrated external electrodes to control fluid flow; and

(3) applying a voltage difference to the longitudinal electrodes, thereby causing electrophoretic migration of the species to occur.

14. The process of claim 13, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.

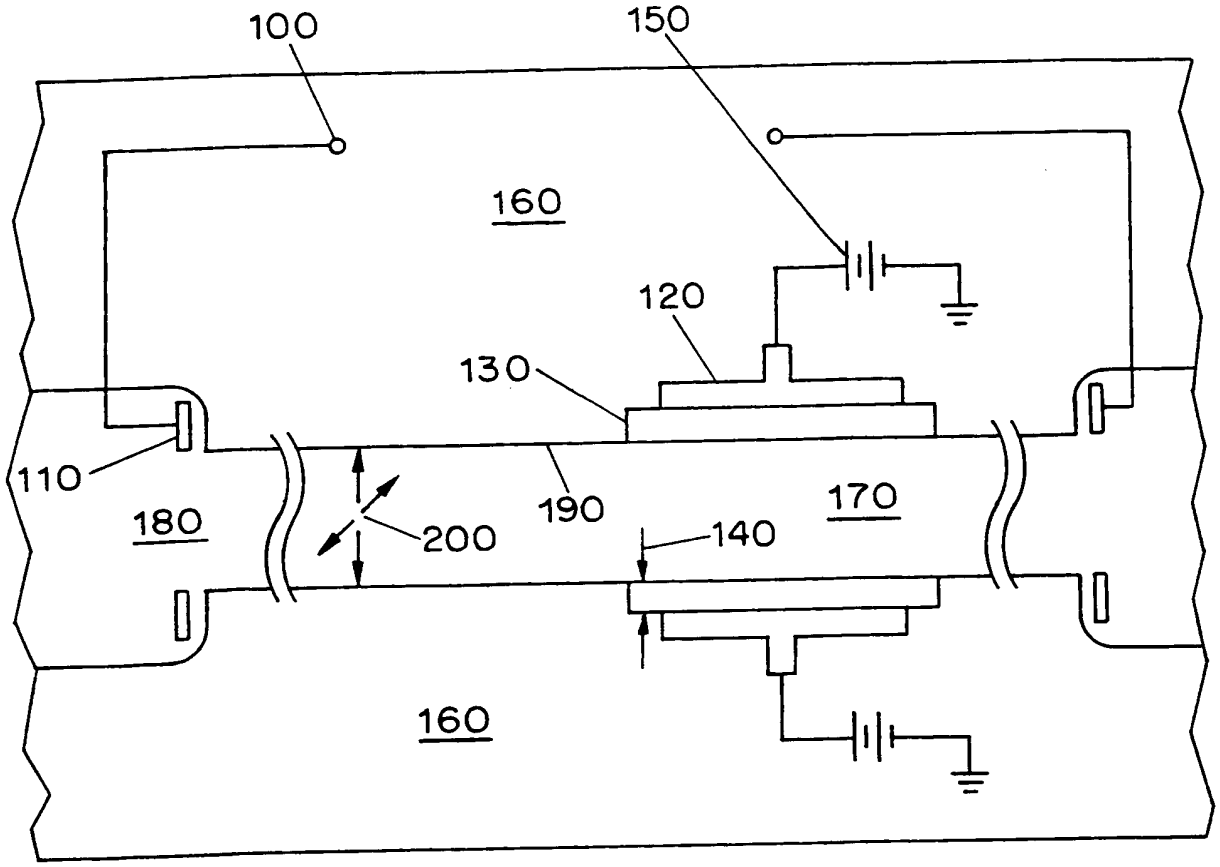
15. A fluid flow process using the device of claim 1, comprising the steps of:

(1) introducing a fluid into the capillary channel;

(2) applying a voltage of less than about 2000 volts to the integrated external electrodes to control fluid flow; and

(3) applying a voltage difference to the longitudinal electrodes, thereby causing fluid flow to occur.

16. The process of claim 15, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.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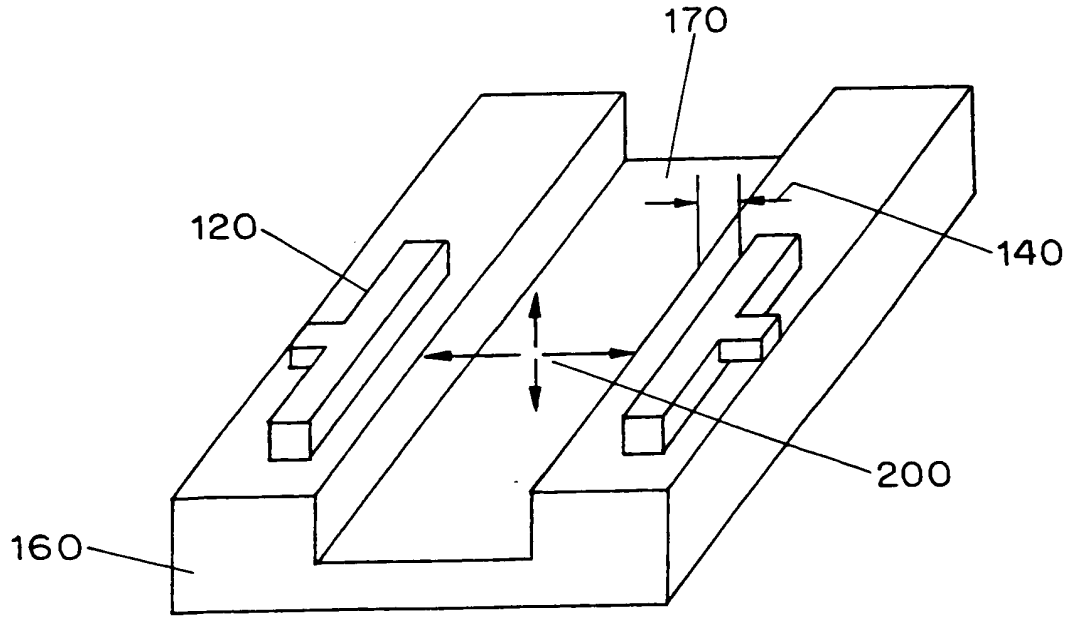
150
160
120
130
110
180
200
190
170
140
160

FIG. 1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200
160

FIG. 2

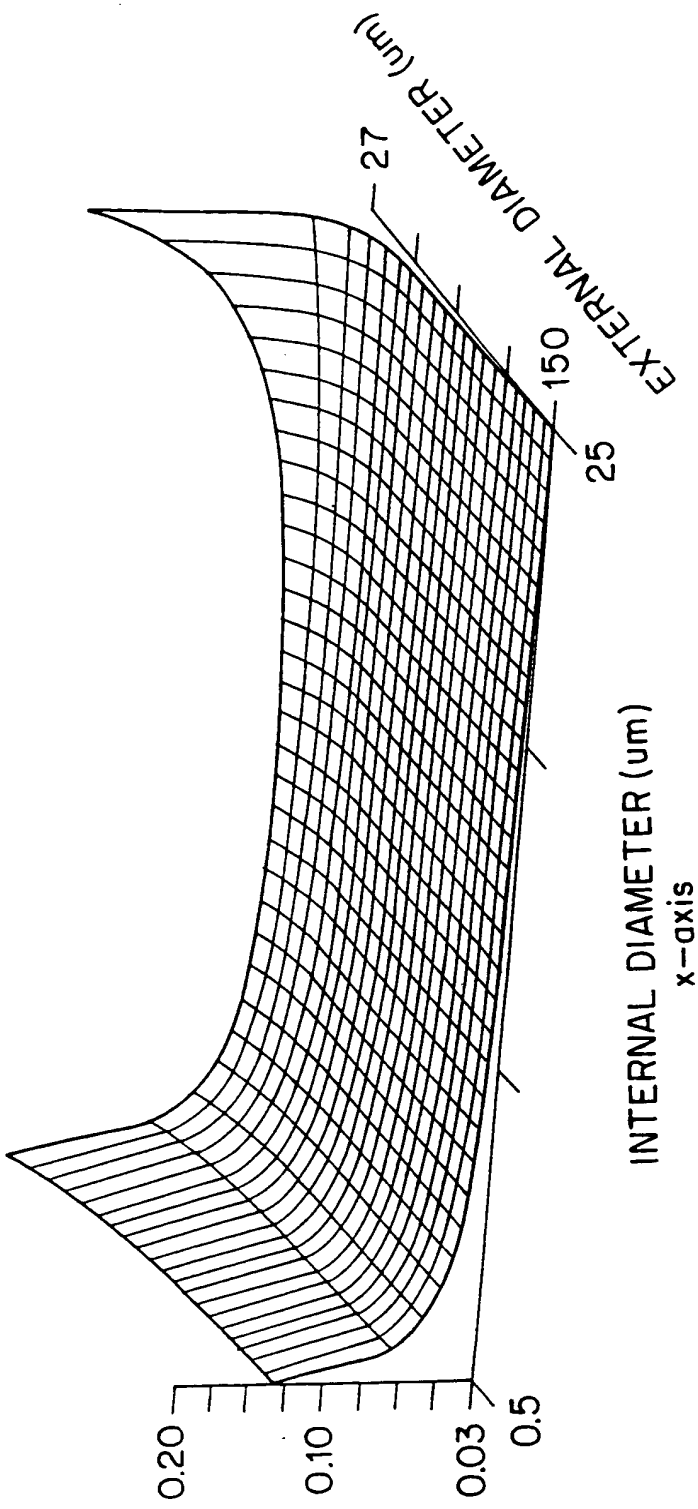
EXTERNAL DIAMETER (um)
27
25
150
INTERNAL DIAMETER (um)
x-axis
0.20
0.10
0.03
0.5
y-axis
INNER SURFACE CHARGE
DENSITY (e.s.u./nm2)

FIG. 3

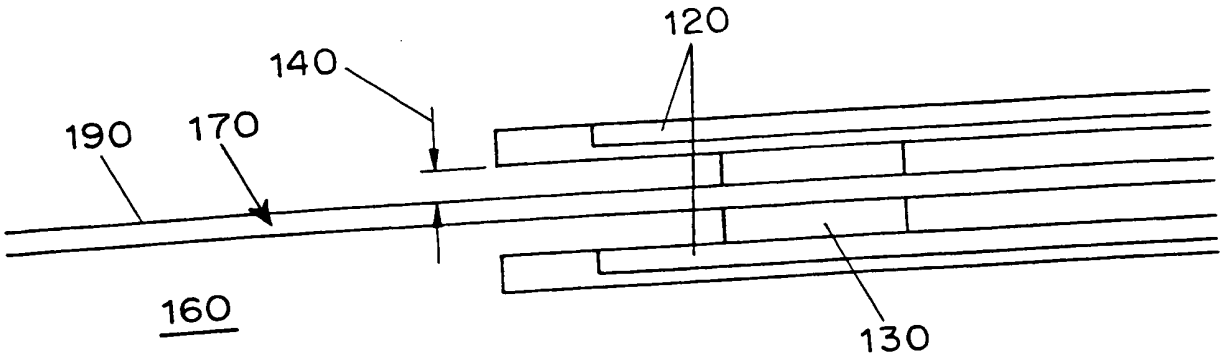
120
140
190
170
160
130

FIG. 4

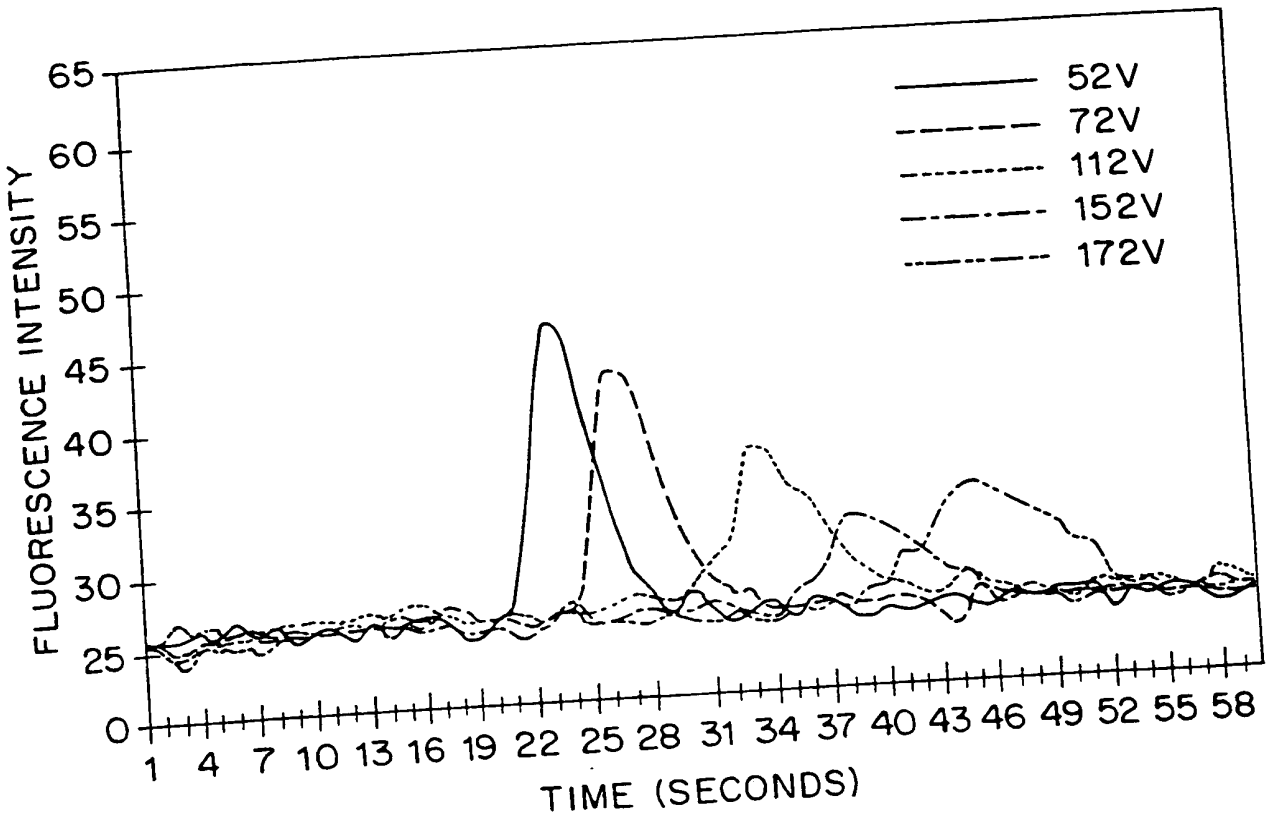
52V
72V
112V
152V
172V
FLUORESCENCE INTENSITY
65
60
55
50
45
40
35
30
25
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
TIME (SECONDS)

FIG. 5

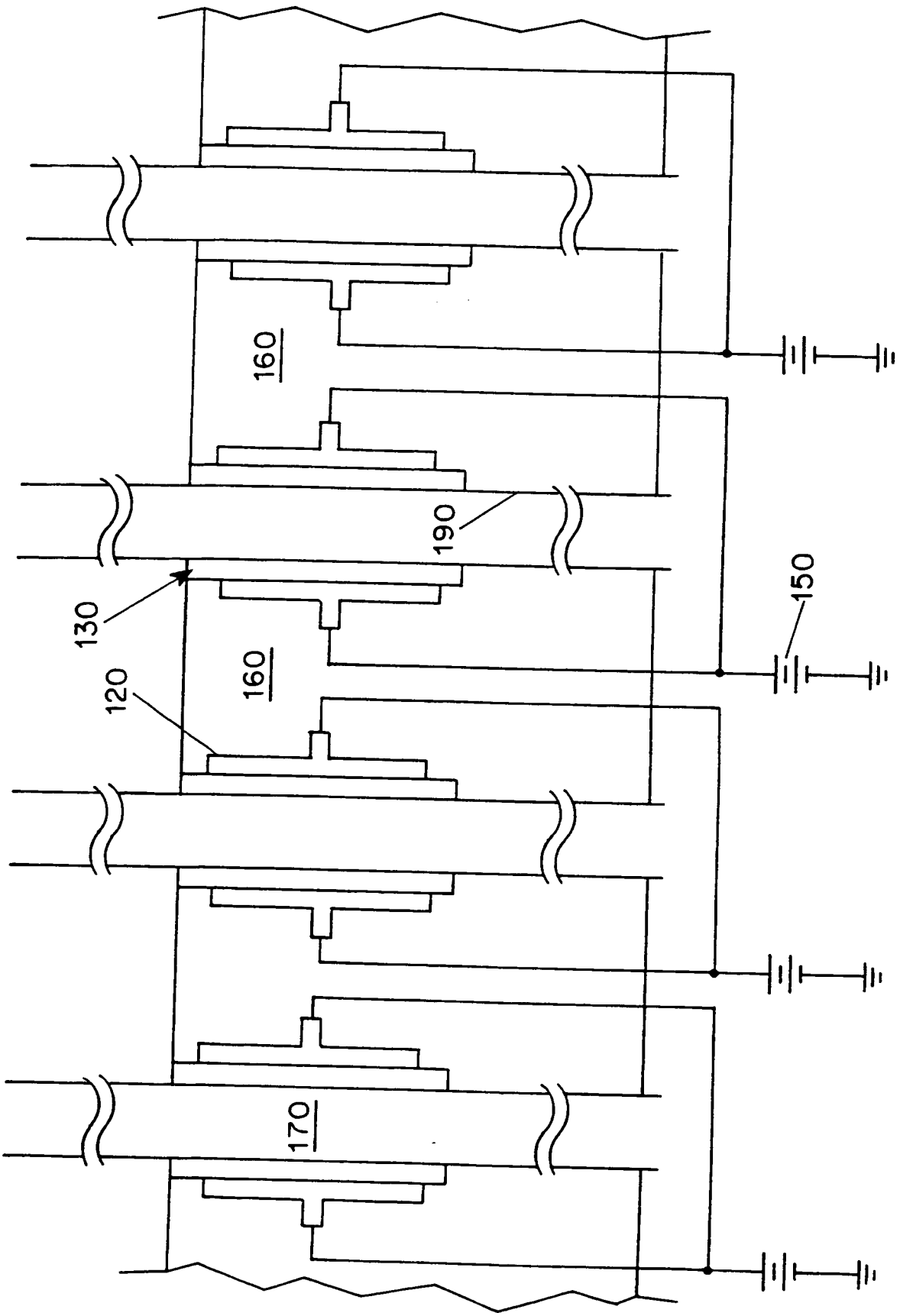

FIG. 6

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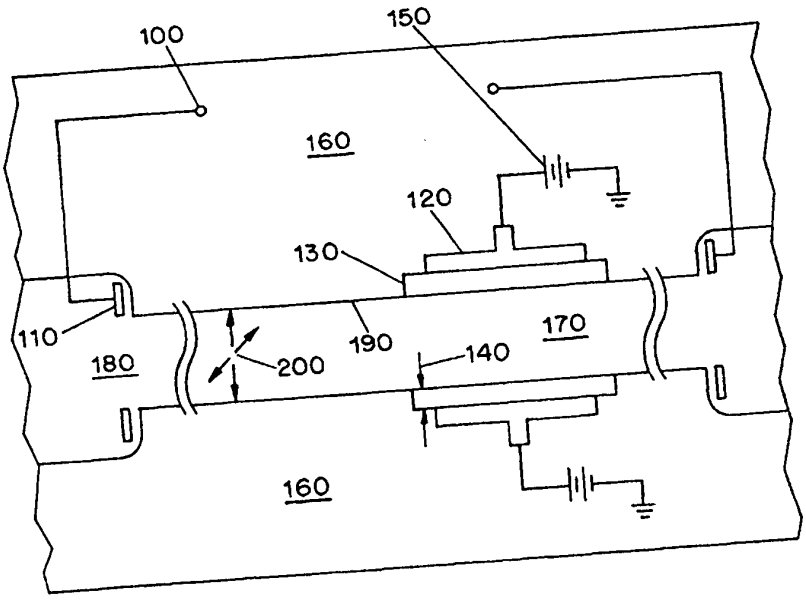
(54) Title: PRACTICAL DEVICE FOR CONTROLLING ULTRASMALL VOLUME FLOW



(57) Abstract

A device for control of ultrasmall volume fluid flow used in the fields of electrophoretic separation, chemical analysis, and microchemical reactions has a substrate defining a capillary channel and integrated external electrodes to control electroosmotic flow. The channel geometry and integrated external electrode proximity reduce the voltage required for control of flow. Longitudinal electrodes provide electrophoretic separation of components. High dielectric material between the integrated external electrode and capillary reduces the voltage required for the control of flow. Real–time flow monitoring and capillary channel surface coating enhance the control of flow.

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AMENDED CLAIMS
[received by the International Bureau on 12 May 2000 (12.05.00);
new claims 17-19 added; remaining claims unchanged (2 pages)]

14. The process of claim 13, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.

15. A fluid flow process using the device of claim 1, comprising the steps of:

(1) introducing a fluid into the capillary channel;

(2) applying a voltage of less than about 2000 volts to the integrated external electrodes to control fluid flow; and

(3) applying a voltage difference to the longitudinal electrodes, thereby causing fluid flow to occur.

16. The process of claim 15, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.

17. A device for performing fluid flow having an efficiency factor Γ, comprising:

a substrate defining a capillary channel, wherein the capillary channel comprises an inner wall surface, first and second ends, and a cross section of less than about 200×10^{-9} square meters; and

at least one integrated external electrode spaced apart from the inner wall surface of the capillary channel by a distance d of less than about 160×10^{-6} meters, wherein the integrated external electrode is positioned to provide a perpendicular voltage field to the capillary channel; and

first and second longitudinal electrodes, the first longitudinal electrode being positioned at the first end of the capillary channel and the second longitudinal electrode being positioned at the second end of the capillary channel, wherein the longitudinal electrodes are positioned at the immediate ends of the capillary channel and are positioned to provide a longitudinal voltage field selectively through the capillary channel, wherein the efficiency factor Γ is greater than 2×10^{-6} $(cm^2/Vs)/(V/micrometer)$.

18. The device of claim 17, in which the efficiency factor Γ is greater than 5×10^{-6} $(cm^2/Vs)/(V/micrometer)$.

19. The device of claim 17, in which the efficiency factor Γ is equal to or greater than 3.3×10^{-5} $(cm^2/Vs)/(V/micrometer)$.

AMENDED CLAIMS
[received by the International Bureau on 12 May 2000 (12.05.00);
new claims 17-19 added; remaining claims unchanged (2 pages)]

14. The process of claim 13, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.

15. A fluid flow process using the device of claim 1, comprising the steps of:

(1) introducing a fluid into the capillary channel;

(2) applying a voltage of less than about 2000 volts to the integrated external electrodes to control fluid flow; and

(3) applying a voltage difference to the longitudinal electrodes, thereby causing fluid flow to occur.

16. The process of claim 15, wherein the voltage applied to the integrated external electrodes is less than about 200 volts.

17. A device for performing fluid flow having an efficiency factor Γ, comprising:

a substrate defining a capillary channel, wherein the capillary channel comprises an inner wall surface, first and second ends, and a cross section of less than about 200×10^{-9} square meters; and

at least one integrated external electrode spaced apart from the inner wall surface of the capillary channel by a distance d of less than about 160×10^{-6} meters, wherein the integrated external electrode is positioned to provide a perpendicular voltage field to the capillary channel; and

first and second longitudinal electrodes, the first longitudinal electrode being positioned at the first end of the capillary channel and the second longitudinal electrode being positioned at the second end of the capillary channel, wherein the longitudinal electrodes are positioned at the immediate ends of the capillary channel and are positioned to provide a longitudinal voltage field selectively through the capillary channel, wherein the efficiency factor Γ is greater than 2×10^{-6} $(cm^2/Vs)/(V/micrometer)$.

18. The device of claim 17, in which the efficiency factor Γ is greater than 5×10^{-6} $(cm^2/Vs)/(V/micrometer)$.

19. The device of claim 17, in which the efficiency factor Γ is equal to or greater than 3.3×10^{-5} $(cm^2/Vs)/(V/micrometer)$.